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Multivalent mannose-decorated NIR nanoprobes for targeting pan lymph nodes

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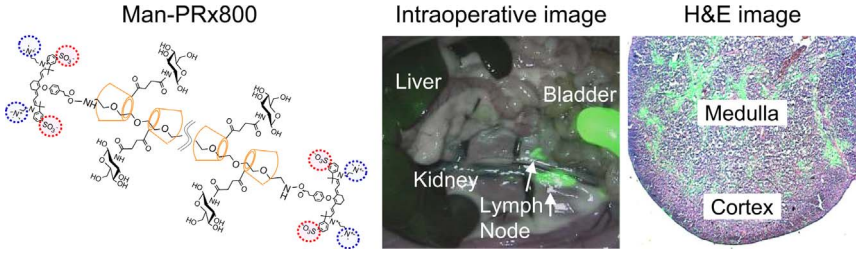
HIGHLIGHTS

- NIR fluorescent nanoprobes to target macrophages in the lymph node.
- Renal clearable nanoprobes enhance the SBR of target tissue.
- Intraoperative pan lymph nodes mapping is demonstrated.
- Surgeons can perform lymphadenectomy with ease and safety using NIR.

GRAPHICAL ABSTRACT



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ABSTRACT

Lymphadenectomy is a prerequisite for most malignancies to define the precise staging of cancer, as well as resect the possible metastases completely. While it improves prognosis, lymphadenectomy often causes postoperative edema or bleeding because of unclear surgical margins. In this study, we synthesized near-infrared (NIR) fluorescent nanoprobes with conjugating various mannose moieties on the surface to target macrophages in the lymph node. Armed with these NIR nanoprobes, we demonstrated the feasibility of intraoperative pan lymph nodes (PLN) mapping and real-time optical imaging under the NIR fluorescence imaging system. We found that even single mannose-conjugated ZW800-1 showed specific uptake in lymph nodes within 4 h, and multiple mannose-employed polyrotaxanes highlighted PLN efficiently with low background signals in major organs. This technology can help surgeons perform lymphadenectomy with ease and safety by identifying all regional lymph nodes proficiently after a single intravenous injection of NIR nanoprobes.

1. Introduction

Lymph node metastasis is an important prognostic factor in many solid malignancies. Diagnostic, prophylactic, or curative lymphadenectomy is usually performed as a standard procedure during the cancer surgery [1–3]. Since the distribution and extent of regional lymph nodes varies in each malignancy, lymphadenectomy often requires that surgeons have advanced techniques; as a result, the quality of lymphadenectomy in each surgery is mostly dependent on a surgeon's skill. Furthermore, this procedure sometimes causes serious postoperative complications such as postoperative edema and bleeding, lymphatic fistula, and tissue injury [4].

Previously, we reported various near-infrared (NIR) fluorophores for mapping pan lymph nodes (PLN) and sentinel lymph nodes (SLN),

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and performed lymphadenectomy in pigs and humans with real-time intraoperative navigation [5–7]. A small molecule NIR fluorophore ZW800-3C was recently used for PLN mapping with improved accumulation and retention in lymph nodes compared to quantum dot-based NIR fluorophores [4,8]. Unfortunately, ZW800-3C largely accumulates in the liver and often results in confusion of lymph node imaging in the abdomen [9]. ZW800-1, however, is a zwitterionic heptamethine indocyanine fluorophore with showing minimal binding on serum proteins or cell surface [10,11]. Preclinical evaluations of toxicity and scale-up chemistry were successfully finished, and clinical trials are ongoing, so ZW800-1 could be clinically available in the near future [10–12]. In addition, it shows excellent optical properties, including a high extinction coefficient, high quantum yield, and excellent optical stability [10]. Therefore, ZW800-1 has been used for synthesizing novel receptor-targeted NIR fluorophores by conjugating highly specific targeted ligands including small molecules, peptides, proteins, and antibodies [10–16].

In order to target lymph nodes, many trials have been made to use mannose receptors because they express on the surface of macrophages and dendritic cells in the lymph node [17–19]. Lymphoseek is the most broadly used radiotracer for SLN mapping, which includes dextran and multiple units of mannose and diethylene triamine pentaacetic acid [20–22]. Nevertheless, this theory has never been translated into mapping all regional lymph nodes via single intravenous administration. In this study, we designed various mannose-conjugated nanoprobes and exploited the feasibility of PLN mapping under real-time NIR fluorescence imaging. Various numbers of mannose moieties were employed on small molecules and polymeric nanoparticles to verify their targeting efficiency to lymph nodes in terms of size, lipophilicity, charge, shape, and flexibility.

2. Results

2.1. Preparation of mannose-conjugated nanoprobes

We prepared the mono- and multivalent mannose-conjugated NIR fluorophores by conjugating mannose moiety to ZW800-1, ZW800-3C, bovine serum albumin (BSA), and polymers such as dextran (DEX), polyacrylic acid (PAA) and polyrotaxane (PRx) (Fig. 1). The chemical profiles of each mannose conjugates are shown in Table 1. For single mannose nanoprobes, an amino-mannose was conjugated to the NHS ester activated NIR fluorophores such as ZW800-1 and ZW800-3C (Supplementary Methods). The conjugation yield was > 92%, and the final products showed > 98% purity after prep-HPLC purification (Figs. S1–S3) [11,23]. To improve PLN targeting with higher receptor binding on macrophages, we introduced multiple mannose moieties on various biomolecules: 1) BSA was used as a model globular-structured protein, 2) DEX is a clinically available semi-globular structured biomolecule and the main composition of Lymphoseek, 3) PAA is a linear polymer with high negative charges on the surface, and 4) PRx is a supramolecular structure, in which many α-cyclodextrins (α-CDs) are threaded onto the polyethylene glycol (PEG) chain. Since the overall hydrodynamic diameter (HD) of Lymphoseek is about 6 nm (mainly composed of 10 kDa dextran and about 25 mannose units), we prepared our nanoprobes based on this commercial product in terms of the number of mannose units, MW and HD. The final HD of multivalent mannose-conjugated nanoprobes are found to be 5–7 nm (Table 1, Fig. S4). All biomolecules contain ZW800-1 for tracking their behavior in the body.

2.2. PLN mapping of monovalent mannose-conjugated ZW800-1 and ZW800-3C

As shown in Fig. 2a, hydrophilic and zwitterionic ZW800-1 (logD = −3.35, net charges = 0) shows virtually no uptake in any lymph nodes in the rat model, while lipophilic and positive charged ZW800-3C (logD = −1.63, net charges = +2) highlights all regional lymph nodes after a single intravenous injection. By introducing a single mannose moiety on nonsticky ZW800-1, Man-ZW800-1 (logD = −2.99, net charges = +1) targeted pan lymph nodes within 4 h post-intravenous injection ($^{**}P < .005$; Fig. 2b). Mannose conjugation on lipophilic ZW800-3C resulted in decreased logD at pH 7.4 (−5.40), but increased background uptake in major organs including liver, spleen, and cartilages (Fig. 2c). In addition, the lymph node uptake was not improved significantly ($P > .05$). There is no significant differences in signal-to-background ratio (SBR) of lymph nodes against muscle among Man-ZW800-1, ZW800-3C, and Man-ZW800-3C (Fig. 2b), but the background signals in major organs of zwitterionic Man-ZW800-1 injected rats were significantly lower than those from both ZW800-3C and Man-ZW800-3C injected animals (Fig. 2c). The nonspecific uptake of Man-ZW800-3C in liver ($^{**}P < .005$), spleen ($^{***}P < .0005$) and kidneys ($^{***}P < .0005$) were significantly higher compared with other tissues/organs due to the positive surface charges.

2.3. PLN mapping of multivalent mannose-conjugated nanoprobes

To confirm the effectiveness of mannose conjugation on different biomolecules, 25 mannose moieties were conjugated on PAA, DEX, and PRx with varying net surface charges, shape, and flexibility (Table 1, Figs. S4–S6). BSA was used as a protein control with employing only 10 mannose units due to the limited surface area, while ZW800-1 was used as a negative control. Man-BSA800, Man-DEX800, and Man-PRx800 showed high regional lymph node signals (Fig. 3a): Globular structured Man-BSA800 was found in neck and lumbar lymph nodes, and semi-globular structured Man-DEX800 was specific to mesenteric lymph nodes. Linear and rigid Man-PAA800, however, exhibited weak uptake for most lymph nodes. Flexible Man-PRx800 was found from all regional lymph nodes including axillary, inguinal, mesenteric and lumber areas compared with all other groups with significant difference ($^{**}P < .005$; Fig. 3b).

Background signals from the surrounding tissues/organs of lymph nodes are important to determine the SBR of lymph nodes. As shown in Fig. 3c, Man-BSA800, Man-DEX800, and Man-PAA800 exhibited high nonspecific uptake in the skin and muscle as well as major organs such as liver or kidneys. Man-PRx800 showed minimal to no fluorescence signals in normal tissues and organs except for nominal liver uptake, which contributed to the high SBR of most lymph nodes. The resected lymph nodes were applied for histopathological analysis combined with NIR fluorescence microscopy. As shown in Fig. 3d, the neck lymph node resected from the rat injected with 100 nmol of Man-PRx800 represented the major uptake in the medulla region, where macrophages overexpress mannose receptors [19].

3. Discussion

SLN biopsy is the standard of care for some of malignancies such as breast cancer and malignant melanoma [1–3]. Likewise, prophylactic regional lymphadenectomy is of significant importance in most malignancies to provide precise staging and to predict the prognosis [5]. Furthermore, curative lymphadenectomy can improve the prognosis of many cancer patients with lymph node metastasis [5]. However, numerous complications accompanied by the lymphadenectomy such as postoperative edema, postoperative bleeding, lymphatic fistula, and tissue injury are significant drawbacks [4].

Under the intraoperative NIR fluorescence imaging system, mono- or multivalent mannose-targeted nanoprobes enable to highlight all lymph nodes in the whole body after a single intravenous injection. Of note, the success of imaging and surgery is largely dependent on strong signals from the lymph nodes as well as low uptake in the surrounding tissues and major organs, which together decide the relative SBR [24,25]. For example, as shown in Fig. 2, ZW800-3C, Man-ZW800-1, and Man-ZW800-3C showed similarly high uptake in PLNs, but only zwitterionic Man-ZW800-1 displayed minimum to no signals in the